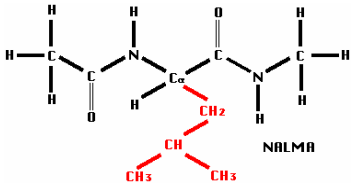
Hydration is important

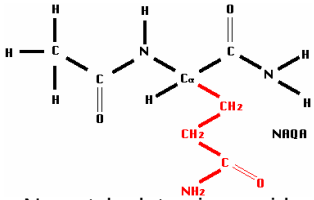
The importance of the solvent in protein folding and some of its effects are well recognized, for example in the formation of a hydrophobic core. Significant long range forces, attributed to the energy required to reorganize the hydrogen bonding network of the solvent as surfaces come closer together, have been observed in the association of lipid bi-layers [1] and protein complexes such as collagen fibers [2]. The "hydration force" is distance dependant and may be repulsive or attractive depending on the chemical nature of the surfaces. What is less well known is the strength and length scales of interactions involving hydration forces when the surfaces are on the size scale of segments of secondary structure or individual amino-acid side chains. Directly measuring the role of hydration in protein folding, dynamics and ligand binding has proven to be difficult and experimental techniques which probe hydration forces are under-represented.

Our approach to understanding hydration

We have developed a model-systems approach which allows us to investigate hydration forces in protein folding [2,3]. Aqueous solutions of blocked amino-acids, of the type shown in Figure 1, are prepared at various concentrations. The dilute solutions are a model of early stages in folding when amino acids are relatively solvent exposed, while the concentrated solutions are a model of the late stages of folding when not enough water is available to provide each amino acid with its own solvation shell. X-ray diffraction patterns are collected from each (Figure 2). Scattering from liquids



N-acetyl leucine methyl amide



N-acetyl glutamine amide

Figure 1.

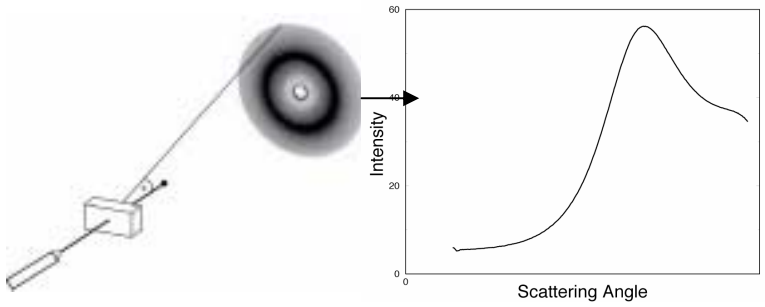
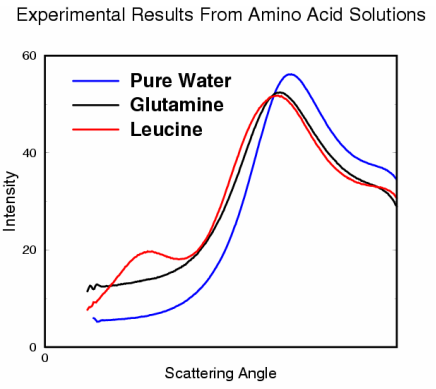
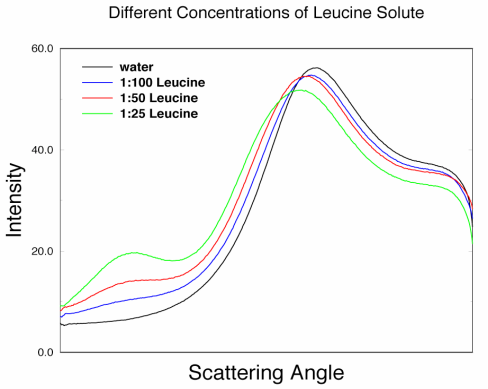


Figure 2.

results in diffuse rings which can be represented as one-dimensional scattering curves, following circular integration. Our results showed that the scattering curves for solutions containing the hydrophobic amino-acid leucine were different from the hydrophilic amino-acid glutamine despite their similar size and scattering power (Figure 3). In the case of leucine, a diffraction peak corresponding to an effective Bragg spacing of 8Å grows in size with increasing concentration, does not shift position (Figure 4) and suggests a stable arrangement of molecules that increases in abundance with increased concentration. Computer simulations of the same systems were carried out by our group. The simulation results showed qualitative trends corresponding with the experiment (Figure5). The experiments, on their own, are ambiguous in the sense that precise molecular arrangements are difficult to infer, while the simulations require experimental verification of their accuracy. The combination of the two allow us to interrogate stabilized structures in the solvent environment.





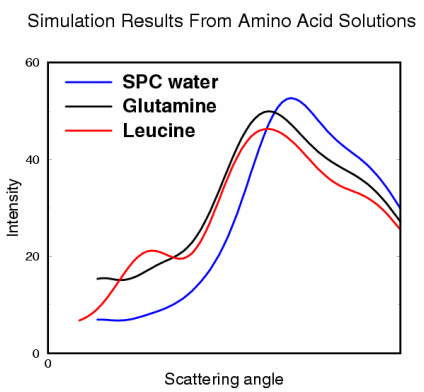


Figure3. Figure 4. Figure 5.

Our most concentrated leucine simulations of one solute for 25 molecules of water model a stage in folding where the hydrophobic core has collapsed with few waters of hydration. The experiments do not support a configuration of leucines in a cluster, excluding water. Rather, dispersed and small aggregates of leucines sharing hydration shells, show greater consistency with experimental results (Figure 6). Our work demonstrates that solvent separated hydrophobic amino acids are stabilized to a significant extent with respect to configurations of hydrophobic groups in direct contact. Work by Pande and Rokhsar [4] further supports the importance of a solvent separated minimum in protein folding as they have observed a solvent stabilized expanded hydrophobic core as a stable point in their simulation of the unfolding of a β-hairpin. In addition, the stability of molten globules in general may rely, to a significant extent, on interplay between solvent and hydrophobic groups. We intend to use solution scattering coupled with simulation to investigate a molten globule system as described below.

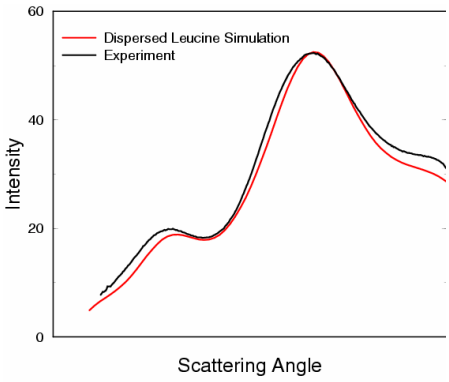


Figure 6.

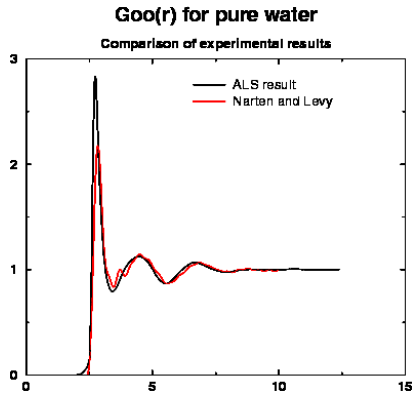


Figure 7.

Developing an accurate description of water

Many water models exist, differing in the amount of detail used to describe water interaction potentials and accuracy with which they agree with various experiments. One reason for the proliferation of water models, which differ in agreement with experiment, is the lack of consistency in experimental results, particularly in the determination of the radial distribution function of water. The radial distribution function describes the average structure of liquid water on a molecular scale. Using x-ray liquid scattering we have extracted our own radial distribution function for ambient-temperature water. We have shown that our results[6,7] are an improvement over the previous results in an experimental sense because of the well characterized synchrotron source (Advanced Light Source), higher quality detector, and a consistency check made possible by the use of an area detector rather than single point detector. We also made theoretical improvements in our extraction of the radial distribution function from our x-ray experiment in that we have taken into account chemical bonding effects in our calculation of the atomic form factor of water. Our result differs significantly from the most commonly cited experimental radial distribution function (Figure 7). The water and simulation communities have received our data with great interest.

We are currently in the process of analyzing data collected at the ALS using a different geometry and detector from our previous work. In addition to repeating and further validating our ambient water results we have conducted temperature dependent studies on both pure water and our most concentrated amino-acid solutions (leucine and methionine) ranging in temperature from 15C to 70C. The temperature-dependent pure- water data will be a good test for the generality of water-model potentials, something sorely lacking in the water simulation community. In addition, these results will have implications for protein folding and stability in extremeophiles.

Future work

We have conducted experiments on methionine in anticipation of future experiments on selino-methionine both in monomeric form and perhaps as part of a molten-globule system. The metal, Selenium which replaces the Sulfur of methionine, has an absorption edge that commonly is taken advantage of in x-ray crystallography to solve the phase problem. In our experiment we intend to use its signal to attain an experimentally determined radial distribution function for this hydrophobic amino-acid and calculate a potential of mean-force. This would quantitatively probe the influence of solvent on the association of polypeptides in the later part of the folding pathway.

We would like to explore the structural implication of our model system by making connections to molten globules. The stability of a molten globule may rely heavily on the effects of the solvent. The system we have chosen to investigate is the molten globule of the helical domain of α-lactalbumin.Wu and Kim[8] have shown that the helical domain of α-lactalbumin continues to have molten globule properties when all hydrophobic residues have been mutated to leucine. We propose to see if the same is true for methionine and therefor selino-methionine. We would use simulations to identify residues which maximize signal when mutated to selino-methionine and create the peptide using either site directed mutagenisis or chemical protein synthesis. Solution scattering experiments will be compared to our computer simulations and probe the degree to which water has infiltrated the hydrophobic core of the molten globule and contributes to stabilizing its expanded state. We would then have a structural model for water in a molten globule. Calculating a radial distribution function for the selino-methionines in the core would provide a potential of mean-force and thus a more direct quantitative measurement of forces involved in protein folding.

References

1. R. P. Rand, V. A. Parsegian, (**1989**) “Hydration forces between phospholipid bilayers” *Biochimica et Biophysica Acta.* **988**: 351-376.
2. S. Lieken, D. C. Rau, V. A. Parsegian, (**1994**) "Direct measurement of forces between self-assembled proteins: Temperature-dependent exponential forces between collagen triple helices." *Proc. Natl. Acad. Sci.* **91**:276-280.
3. J. M. Sorenson, G. Hura, A. K. Soper, A. Pertsemlidis, T. Head-Gordon, (**1999**) "Determining the role of hydration forces in protein folding." *J. Phys.Chem. B* **103**:5413-5426. (Feature Article and cover)
4. G. Hura, J. M.Sorenson, R. M.Glaeser, T. Head-Gordon, (**1999**) "Solution x-ray scattering as a probe of hydration dependant structuring of aqueous solutions." *Perspectives in Drug Discovery and Design* , **17**: 97-118.5. V. Pande & D. Rokhsar (**1999**). "Molecular dynamics simulations of the unfolding and refolding of a β-hairpin." *Proc. Nat. Acad. Sci.* **96**:9062-9067.
6. A. H. Narten & H. A. Levy (**1971**) "Liquid water: molecular correlation from x-ray diffraction"*J. Chem.Phys.***55**:2263-2269.
7. G. Hura, J. M. Sorenson, R. M. Glaeser, T. Head-Gordon, "A quality x-ray scattering experiment on liquid water at ambient conditions." *J. Chem Phys.* **113**, 9140-9148.
8. J. M.Sorenson, G. Hura, R. M. Glaeser, T. Head-Gordon, "What can x-ray scattering tell us about the radial distribution function of water." *J. Chem Phy.* **113**, 9149-9161.
9. L. C. Wu & P. S.Kim (**1997**) "Hydrophobic sequence minimization of the α–lactalbumin molten globule." *Proc. ` Natl. Acad. Sci.*. **94**:14314-14319.